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PERSPECTIVE

The Future of Layer-by-Layer Assembly: A Tribute to *ACS Nano* Associate Editor Helmuth Möhwald

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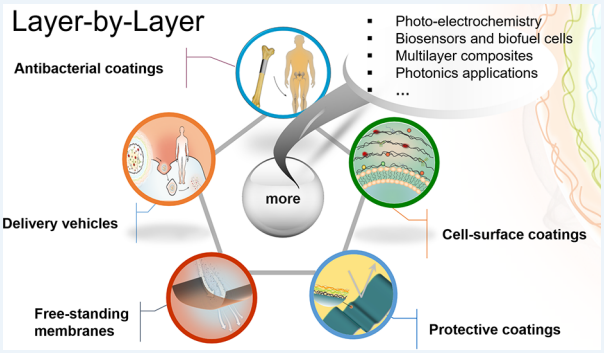
ABSTRACT: **Layer-by-layer (LbL) assembly is a widely used tool for engineering materials and coatings. In this Perspective, dedicated to the memory of *ACS Nano* associate editor Prof. Dr. Helmuth Möhwald, we discuss the developments and applications that are to come in LbL assembly, focusing on coatings, bulk materials, membranes, nanocomposites, and delivery vehicles.**



The classic realization of layer-by-layer (LbL) assembly was introduced three decades ago,[1−7] with significant contributions from our colleague, the late *ACS Nano* associate editor Helmuth Möhwald.[8−33] Research on Langmuir−Blodgett deposition and later LbL assembly carried out by Helmuth Möhwald[34] created a critically important foundation for development of multilayer composites based on hybrid organic−inorganic nanostructures and numerous related technologies. Early studies in this area involved self-assembly of multilayers from graphite oxide,[35] clay sheets,[36] nanoparticles,[8,37−42] and other materials, serving as conceptual growth points for the evolution of the fields of biomimetic composites, energy materials, and self-assembly. Numerous studies inspired and authored by Helmuth Möhwald not only paved the way for rapid expansion of nanoparticle-based design of nanocomposites, but also led to understanding biomineralization processes in Nature and their utilization in diverse areas of technology.[43] This field has since undergone massive expansion that continues to this day, and LbL is now an established and widely used technique for coating and encapsulation. With several publications per day, LbL assembly has matured from a scientific oddity to an accessible and useful tool for the preparation of nanoscale functional films. It continues to be used to create new commercial products, making it as interesting for various industries now as chemical vapor deposition (CVD) and physical vapor deposition (PVD) were in the 1960s. Whereas the past and the present of LbL have been extensively reviewed,[11,44−50] in this Perspective, we focus on future opportunities using this exciting technique.

The classic realization of layer-by-layer assembly as a dip-and-rinse process has several conceptual advantages over other methods of materials preparation that predicated its wide use in science and technology. First, compared to other techniques, for instance, sequential spin-coating, it enables preparation of nearly ideal conformal coatings on surfaces of any topography. Thus, it has been applied to planar surfaces, spherical particles, inside pores, and onto other more complex geometries.

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Second, LbL is universal and flexible. It is compatible with other chemistries, meaning that a wide variety of different surfaces can be coated, not only charged substrates. The sequential assembly of the layers involves a washing step after the addition of each layer, which reduces the excess non-assembled materials or molecules. Because of the large variety of materials out of which layers can be formed, LbL enables convenient surface chemistry tailoring. Another important characteristic of LbL technology is the broad and independent variability of each double layer, which, in contrast to many other coating and encapsulation technologies, enables the modular construction of multifunctional devices like a box of bricks that have different properties and can be combined in different ways. These properties, combined with the availability of various stimuli[51−55] to control responsiveness of polyelectrolyte assemblies, make LbL an extremely versatile technology platform. Third, the LbL method replicates the essential aspects of physics and chemistry of materials engineering in living organisms, and therefore, it leads to the amazing spectrum of biomimetic materials. Importantly, they may or may not be based on biomacromolecules pertaining to a specific biological process. Replicating the molecular-scale adaptation of the different structural components at the interfaces taking place in, for instance, biomineralization, one can attain structures and properties equal to or better than those of materials found in biology.[56] Note, however, that as with biology, many LbL processes require time for atomistic relaxation at the interfaces. Although the formation of multilayers approaching thermodynamic equilibrium is a rather time-consuming process, many future technologies will require such highly complex structures. This complexity will be illustrated below for some especially promising developments in this field. Of note are several successful reports of accelerating LbL processes using automated procedures on both planar and colloidal templates.[57−61] In parallel, endeavors have also been undertaken to produce coatings with properties similar to those of LbL films but using single-step approaches.[62−64]

In this Perspective, we highlight future directions with a focus on three areas: (1) functional **coatings** on planar and highly curved surfaces, (2) free-standing **membranes** and **bulk materials**, and (3) delivery vehicles based on **encapsulation** for biomedical applications.

LAYER-BY-LAYER-BASED FUNCTIONAL SURFACE COATINGS

Versatility of Coatings. Layer-by-layer coatings are extremely versatile. They enable variability in (1) composition, *i.e.*, the integration of different materials; (2) vertical structuring normal to the surface, *i.e.*, the possibility to create defined

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sequences of layers;[65] and (3) anisotropic alignment, *i.e.*, to orient anisotropic materials within layers.

(1) Toward Multinanocomposites. Among all methods for functionalizing surfaces, LbL assembly arguably has the largest choice of deployable components (inorganic salts, organic molecules, polymers, DNA,[66,67] graphene oxide, biomolecules, lipids, nanoparticles, or biological objects including cells[68]). In polyelectrolyte multilayers, one can bring tens (if not hundreds) of materials together in ordered ways, whereas the number of components in most current nanocomposites is less than ~20. The precision of LbL in controlling the structures of materials bridging the molecular, nano-, meso-, and microscales[69−72] makes it possible to create conformal coatings with exceptionally high curvatures, including functionalized particles,[73−76] and to design self-assembled nanocomposites with previously unexpected combinations of macroscale properties.[36,69,77] Along these lines, LbL makes it possible to demonstrate the transition between nano- and macroscale optical effects in gold films experimentally by precisely tuning interparticle distances in multilayers of silica-coated gold nanoparticles,[78,79] leading to extremely efficient substrates for surface-enhanced Raman scattering (SERS) detection.[80] The fundamental findings regarding rare combinations of properties made using LbL-based composites were confirmed using other techniques, such as vacuum-assisted filtration[81−83] and spin-coating.[84] Layer-by-layer assembly enables the design and preparation of materials with adjustable multifunctionality, which is difficult, if not impossible, using other formulation technologies. Thus, LbL offers the tools to fabricate advanced materials by combining heterogeneous components with potential applications in optoelectronic devices, smart surfaces, solar cells, *etc.*

(2) Toward Three-Dimensional (3D) Coatings. The LbL technique also offers multiple approaches for the fabrication of composite materials from heterogeneous components, where the compositions of the materials are varied in the direction normal to the substrate. In addition to gradients in chemical composition, one can also vary the mechanical, optical, and electronic properties of composites in the vertical direction. The LbL concept can also be integrated with other nano- and microfabrication techniques. In particular, the use of printing strategies and combinations with other 3D coatings with variable vertical composition are possible. Combining LbL assembly with other modern strategies (*e.g.*, roll-to-roll, lithography, and 3D printing, *etc.*) as well as high-throughput production methods[57,58,85−88] is beneficial for the preparation of novel functional LbL composites. Note that multilayer nanocomposites, made by LbL and Langmuir−Blodgett deposition, are extensively used in industry already, albeit produced using closely related, derivative methods. Some representative examples of these multilayer composites are those based on various forms of nanocarbons (graphene, graphene oxides, graphene, nanotubes, nanoribbons, graphene carbon quantum dots, *etc.*[81,82,89−91]) and those based on various forms of ceramic nanoplatelets (clay, metal oxides, MXenes, *etc.*[89,92−94]). The former are employed in energy technologies, whereas the latter are used in membrane and coating technologies. In each case, the composite multilayer production is reliable, scalable, and low cost due to self-assembly of anisotropic colloids. Future directions in multilayer biomimetic composites are likely to include computational design of the multilayers starting from molecular dynamics[95−97] and coarse-grained models of the multilayers.[92]

(3) Toward Materials with Complex Anisotropies. Most of the current materials are isotropic. Materials with anisotropic properties are, in general, more difficult to prepare and to characterize. For example, grazing incidence spraying[98] enables alignment of nanowires, nanorods, and nanofibers in plane[99,100] during the deposition of individual layers in LbL films. With unidirectionally oriented multilayers, one can fabricate films containing ultrathin polarizers.[100] This approach, however, is capable of producing more complex anisotropies, even over large surface areas, by changing the direction of alignment in each individual layer of a multilayer film. We are just starting to realize materials with crisscross and even helical superstructures. Materials with such anisotropies are likely to be interesting for various applications in mechanics, photonics, and other areas.

Protective Coatings. Layer-by-layer assembly provides a convenient coating strategy for the protection of consumer products, such as paints for corrosion protection or antigraffiti coatings.[101−104] Here, among the spectrum of technological advances based on LbL materials, one should mention anticorrosion coatings investigated by Möhwald and co-workers. Andreeva *et al.* deposited oppositely charged $(PEI/PSS)_n$ polyelectrolyte (PE) multilayers on aluminum surfaces.[102] The corrosion processes on the aluminum surfaces were blocked due to the pH-buffering ability of polyelectrolyte-based LbL coatings (Figure 1a). Another representative advance in this area is halloysite nanocontainers for anticorrosion coatings. Shchukin *et al.* first deposited LbL-assembled polyelectrolyte multilayers of $(PAH/PSS)_n$ on the surfaces of inhibitor-loaded halloysite nanotubes.[105] After the halloysite nanocontainers were embedded, the sol−gel SiO_x/ZrO_y active composite coatings with the nanocontainers showed long-term anticorrosion performance. The LbL composite multilayers provide effective storage and prolonged release of the inhibitor. Similarly, SiO_2 particles coated with LbL multilayers entrapping inhibitors were used as nanocontainers to achieve self-healing and anticorrosion composite coating simultaneously.[18,103] Li *et al.* also designed a silica/polymer double-walled hybrid nanotube loaded with active molecules for metal corrosion protection.[106] A new generation of anticorrosion coatings that possess passive matrix functionality and that actively respond to changes in the local environment has been introduced.[107] Active corrosion protection aims to *restore* the properties of the material when the passive coating matrix is broken and corrosion of the substrate has started. The main component of the self-healing anticorrosion coatings are capsules in flat layers, which provide controlled release of the corrosion inhibitor on demand and only inside the corroded area (see Figure 1a). This release acts as a local trigger for the mechanism that heals the defects. The LbL assembly approach is an effective tool for the fabrication of the capsule shells, controlling release of the corrosion inhibitor on demand. Layer-by-layer assembly enables the use of various materials as shell components, utilizing weak, mostly electrostatic forces for their assembly. Depending on the nature of the "smart" materials (*e.g.*, polymers, nanoparticles) introduced into the container shell, different stimuli can induce reversible and irreversible shell modifications: pH, ionic strength, temperature, ultrasonic treatment, and electromagnetic fields. The different responses that can be observed vary from fine effects, such as tunable permeability, to more profound ones, such as total rupture of the container shell. These different behaviors depend on the composition of the polyelectrolyte multilayers (*e.g.*, weak polyanion−weak polycation or strong polyanion−weak polycation-based interactions).

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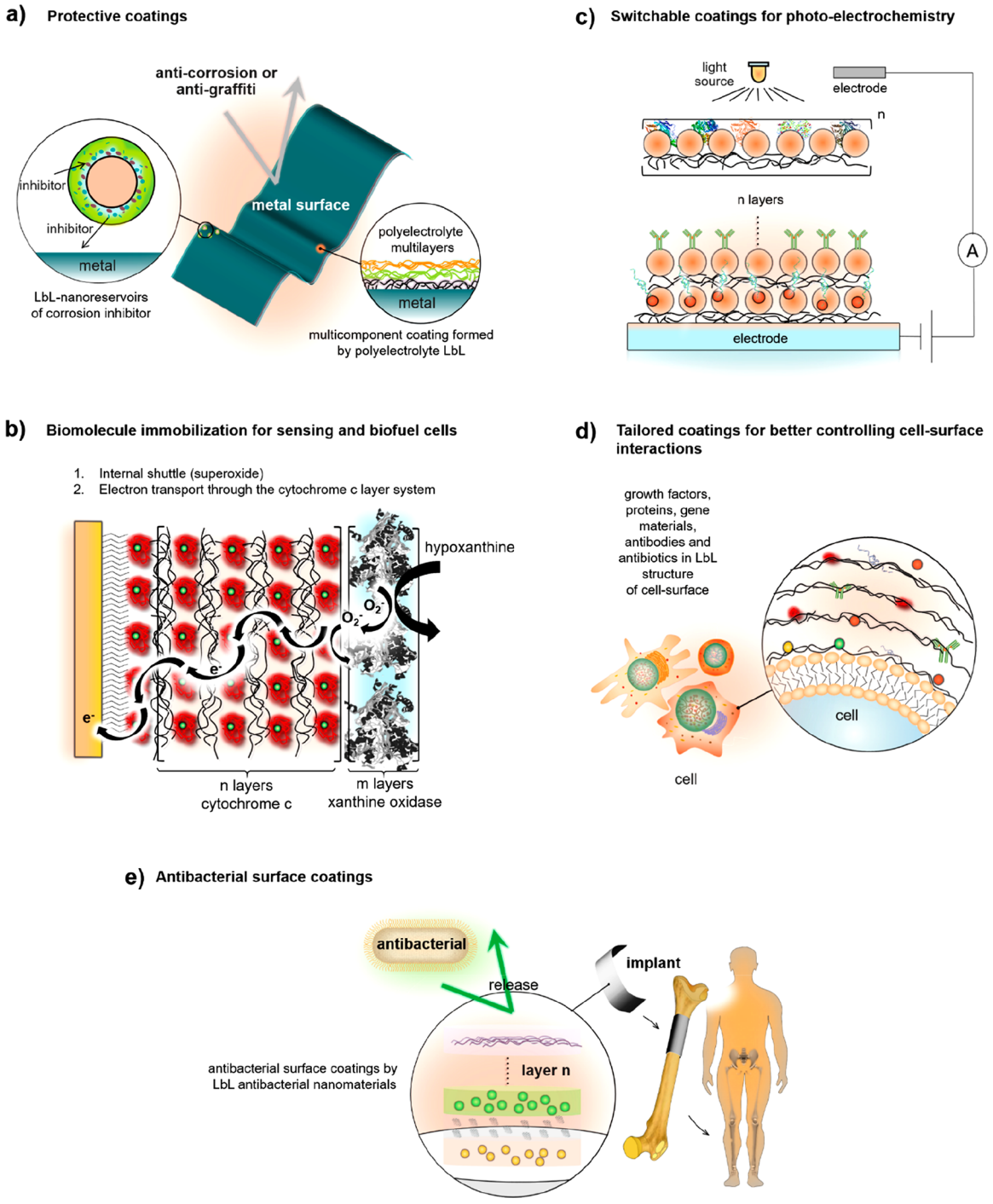

Figure 1. (a) Layer-by-layer assembly can be used in protective coatings in several ways, *e.g.*, as nanoreservoirs of corrosion inhibitors and in multicomponent coatings. (b) Layer-by-layer assembly can be used for biomolecule immobilization in sensing devices and biofuel cells. (c) Layer-by-layer assembly can be used in photoelectrochemical devices to create 3D structures; in medical devices, different biological materials can be assembled in each layer independently. (d) Tailored coatings for better control of cell−surface interactions. (e) Layer-by-layer assembly can be used in antibacterial coatings of implants.

Coatings for Photonics and Energy-Related Applications. There are numerous energy applications that can take advantage of the tunable mechanical, electrical, and chemical properties of LbL composites.[108] In fact, the first implementation of graphene composites on electrodes currently used in a variety of batteries, supercapacitors, conductive inks, and fuel cells was demonstrated in LbL composites referring to these materials as graphite oxide in 1996.[35] The excellent laminar organization of the films also afforded demonstration of the transition from the nonconductive state of graphite oxide to reduced graphene and their utilization in lithium batteries.[109] Composite materials with identical layered design were later produced by other techniques, such as vacuum-assisted filtration, are widely used in the technology.[81] There are also many other energy-conversion devices that employ LbL multilayers from electroconductive materials which include batteries, supercapacitors, catalysts, solar cells, and fuel cells, and these modern applications frequently place higher demands on the performance

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of the composites.[110] Layer-by-layer multilayers can integrate the properties of different constituent materials, and judicious design enables them to take on multiple roles and functionalities, for instance, as battery anodes or ion-transporting membranes.[111−113] Layer-by-layer assembly also facilitates the creation of controlled assemblies to study photonic properties of materials. Layer-by-layer assembly enables composite functional materials that combine polymers with oppositely charged nanoparticles. Such structures can easily be created on planar substrates[114−117] and on colloidal microspheres.[23,118−120] The organized superstructures from semiconductor nanoparticles (NPs), also known as quantum dots (QDs), can also be made using LbL as was demonstrated for CdS, PbS, and TiO_2.[121] The advantage of QDs compared to graphite/graphene oxide is that they are capable of emitting in the visible[114,122] and near-infrared[122] parts of the spectrum; this property has been used to fabricate luminescent self-assembled films and to study energy transfer in such composites.[115] Directed energy transfer from specific layers of QDs toward an interface or electrode was made possible exactly due to the possibility of arranging the LbL layer in the order of decreasing or increasing band gaps in graded semiconductor nanostructures.[123] Excitation recycling in LbL-grown graded band gap QD structures has been demonstrated[116] and ascribed to superefficient exciton funneling to the layer containing the largest QDs.[117] Besides graphene, layer-by-layer assembly is also compatible with other emerging two-dimensional (2D) materials such as hexagonal boron nitride whose LbL coatings yield exceptional performance as gate dielectrics in graphene field-effect transistors.[124] Last but not least, LbL has been used to build coatings for electromagnetic shielding. Flexible and electrically conductive thin films are required for electromagnetic interference (EMI) shielding of portable and wearable electronic devices.[93] The LbL technique enables combinations of nanoparticles and polymers, providing a platform for developing hierarchical architectures with a combination of properties including mechanical strength, transparency, and conductivity.[89] Spin-spray LbL enables rapid assembly of 2D Ti_3C_2 MXene−carbon nanotube (CNT) composite films for EMI shielding. These semitransparent LbL MXene−CNT composite films showed high conductivities and high specific shielding effectiveness, which are among the highest reported values for flexible and semitransparent composite thin films.

Biomolecule Immobilization for Sensing and Biofuel Cells. The LbL technique has found widespread applications in the fixation of biomolecules to surfaces[49,125] because it enables (1) engineering of man-made materials with structural analogy to biomaterials; (2) the controlled deposition of biomolecules because deposition can be governed not only by the number of layers but also by adjusting pH, ion concentration, temperature, and polyelectrolyte and biomolecule concentrations in each layer; (3) the defined integration of different biomolecules in different layers and, thus, the creation of sequential signal chains; and (4) the incorporation of other functional components, such as mediators, which can facilitate electron transfer between immobilized molecules and electrodes or lipids, which, in turn, facilitates integration of more hydrophobic membrane proteins.[126] Furthermore, additional layers on top of the biomolecular assembly enhance the stability of the coatings and ensure efficient discrimination against unwanted species when the multilayer structure is used for sensing purposes. Interestingly, biomolecules cannot simply be passively incorporated into LbL architectures, but because they often carry charges, they can be used as separate building blocks in the assembly process. In this context, alternating polymer/biomolecule or NP/biomolecule structures can be formed,[127,128] as well as pure biomolecular LbL assemblies, such as DNA/protein or protein/protein multilayers.[129] The beauty of the technique can also be demonstrated by immobilizing different biomolecules in different layers on the sensing surface. This localization enables the construction of defined signal pathways by exploiting sequential reaction schemes. Here, reaction products formed in one layer can be further converted in a subsequent layer, as shown in Figure 1b.[13] These artificial architectures can mimic biological functions, such as sequential electron transfer reactions or switchable pathways.[130] This capability enabled, for instance, the first implementation of tissue-adapted neuroprosthetic implants from conductive composites[131] and light-induced excitation of neurons.[132] Further steps in this directions can be based on direct electron transfer between the immobilized protein molecules. The LbL technique enables the artificial arrangement of redox centers, while keeping them in or close to their natural states. Here, developments are still at early stages—more advanced structures appear to be feasible though, such as the arrangement of enzymes into complex cascades to create artificial metabolome structures with high efficiency.[133−135]

Switchable Coatings for Photoelectrochemistry. Electrochemical devices can be controlled by light based on photosensitive switches, such as QDs.[136−139] Light-generated charge carriers can create photo currents, which enables both control and monitoring of electrochemical reactions (see Figure 1c).[140] Inorganic, photoactive materials such as QDs are commonly used in such applications, but light-sensitive biomolecules have also gained considerable interest for the conversion of light into electrical or chemical energy.[141] An example is the protein supercomplex photosystem I, which can be assembled with the help of negatively charged DNA and the positively charged redox-protein cytochrome *c*. This system generates well-defined photocurrents, the magnitude of which depends on the number of deposited layers.[142] Charge transfer in LbL structures has been well-studied by numerous groups.[14,17,127,143−145] Layer-by-layer assembly can be used to increase the coverage of redox active molecules by assembling 3D structures, thereby dramatically increasing the analytical signal, but also improving the signal-to-noise ratio (SNR).[146,147] The response from multilayer structures is significantly enhanced compared to the response from single monolayer-based structures. In addition, different kinds of biological modifications can be introduced to the LbL structures of the photoelectrochemical devices. For example, LbL offers the convenient possibility to immobilize enzymes, thereby controlling redox reactions close to the light switches as fixed on the surface of the electrodes. The porous structures of LbL films enable sub-

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strates to reach the enzymes and cosubstrates or reaction products, such as O_2 or H_2O_2, to reach the light switches.[148−150] In the future, we expect antibodies and DNA to be incorporated as recognition elements into LbL-based structures. Creating defined sequences of antibodies or oligonucleotides within the 3D assemblies is also an important goal.[151,152] There is great potential for photoelectrochemical devices to be developed that can sense multiple analytes in parallel. Layer-by-layer structures can also introduce good biocompatibility by modifying working electrodes in such a way that applications in cell-based detection become possible. The detection of several metabolites will enable more specific studies of cellular activities.[152] Moreover, the biocompatibility and the ease of preparation of LbL-based systems will enable the fabrication of miniature sensors for human uses such as wearable health monitors and portable environmental monitoring devices. In addition, electrochromic coatings can be produced by self-assembly of 2D titanium carbide ($Ti_3C_2T_x$) MXene and gel electrolyte with a visible absorption peak shift from 770 to 670 nm and a 12% reversible change in transmittance with a switching rate of <1 s when cycled in an acidic electrolyte under applied potentials of less than 1 V.[153] The LbL film can act as both transparent conductive coating and active material in an electrochromic device, opening avenues for a number of optoelectronic, sensing, and photonic applications. Hybrid systems prepared by LbL assembly of polyoxometalate clusters and poly(4-vinylpyridine) also show reversible electro- and photochromic behavior.[154,155]

Tailored Coatings for Better Control of Cell−Surface Interactions. For many applications, detailed understanding of the interface between cells and underlying substrates is critical.[156,157] It is well established that LbL assembly offers a means to immobilize different biomolecules on surfaces using mild deposition conditions (see Figure 1d).[158] Layer-by-layer assemblies can integrate plasmids,[159] growth factors,[160] proteins, genetic material, antibodies, and antibiotics directly into the layers or the components can be precomplexed with polyelectrolytes and then assembled as complexes.[161] For such biological components, *e.g.*, for growth factors, their action can be extended in time[162] or triggered by external stimuli, whereas their controlled release can be regulated by barrier layers. Multilayers can be prepared from biocompatible polyelectrolytes and their mechanical properties; wettability, and interactions with proteins and cells, can be fine-tuned by chemical cross-linking, thermal annealing,[163] or the addition of nanoparticles into the assembly (see Figure 2).[164] This strategy enables the availability of biomolecules on surfaces to be controlled.[162,165,166] Imagine chemically identical surfaces (composition, roughness, *etc.*), below which nanoreinforced strata are hidden (*i.e.*, deposited) that enable control of the tensile strength of the interface. Other combinations of surface properties can be deposited on top of cell-culture gels. Such surface engineering would be extremely useful for implants and scaffolds as a means to enhance cell adhesion, mobility, and differentiation. In the long term, there are numerous different ways for the LbL technique to be implemented. For example, they can be used to modify scaffolds and implants to create customized environments and interfaces in tissue engineering. Layer-by-layer assembled surfaces can also be laterally patterned as substrates for the growth of cells. Micropatterned deposition of LbL films has been used to generate architecturally organized cellular structures that better mimic the complex microstructures of tissues in the body. For example, patterned cocultures were generated by sequentially

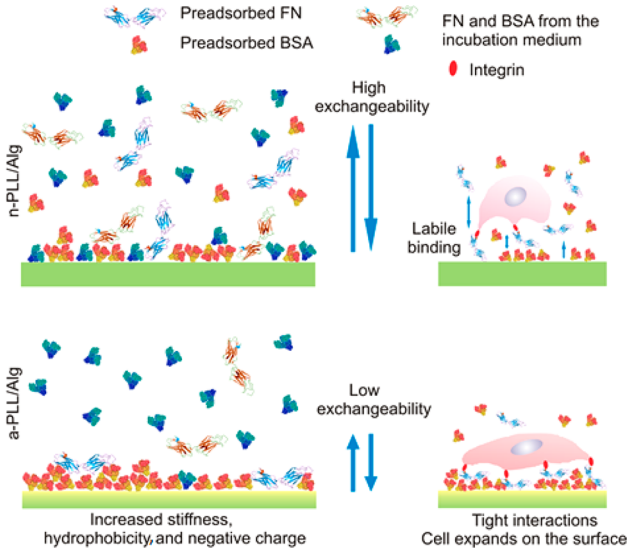

Figure 2. Scheme of the protein adhesion mechanism and the effects on cell adhesion for non-annealed poly(L-lysine/alginate) (PLL/Alg) and annealed-PLL/Alg.[157] Results from the exchangeability assays are schematically described. On annealed PLL/Alg layer-by-layer (LbL) surfaces, proteins exhibit augmented interactions with the substrate, the exchangeability is reduced, and fibronectin (FN), either alone or in cooperation with bovine serum albumin (BSA), has stronger interactions with the LbL surface coating. The effect on cell adhesion is also illustrated.[169] The objects depicted in the scheme are not to scale, and for FN, only the FN III fragment is represented. Adapted with permission from ref 150. Copyright 2019 John Wiley & Sons, Inc.

depositing micropatterned LbL films made of hyaluronic acid and polylysine or collagen that could be used to render regions of a surface adhesive to cells.[167,168] In such cultures, patterned cocultures of liver cells and fibroblasts showed increased functionality compared to various controls.

This strategy ultimately results in a multitude biomimetic composites including those made in bulk form. The diverse composite structures replicated using LbL assembly[170−173] made possible *ex vivo* replication of nacre,[36] enamel,[170] extracellular matrix,[174,175] and models of cellular organelles.[176−178] By combining LbL assembly with other fabrication techniques at the micrometer and millimeter scale, tissue replicas with

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complex geometries have been obtained, such as for bone marrow.[179,180] The exceptional materials properties of the multilayer composites and the generality of the approach have also made possible the design, fabrication, and implementation of implantable devices,[132,181,182] sensors,[53,183,184] drug-delivery vehicles,[185,186] and optical devices,[187,188] exceeding the performance of existing technologies.

Antibacterial Surface Coatings. The advent of LbL films has led to several new strategies for the development of antibacterial coatings, from the fabrication of multilayers with

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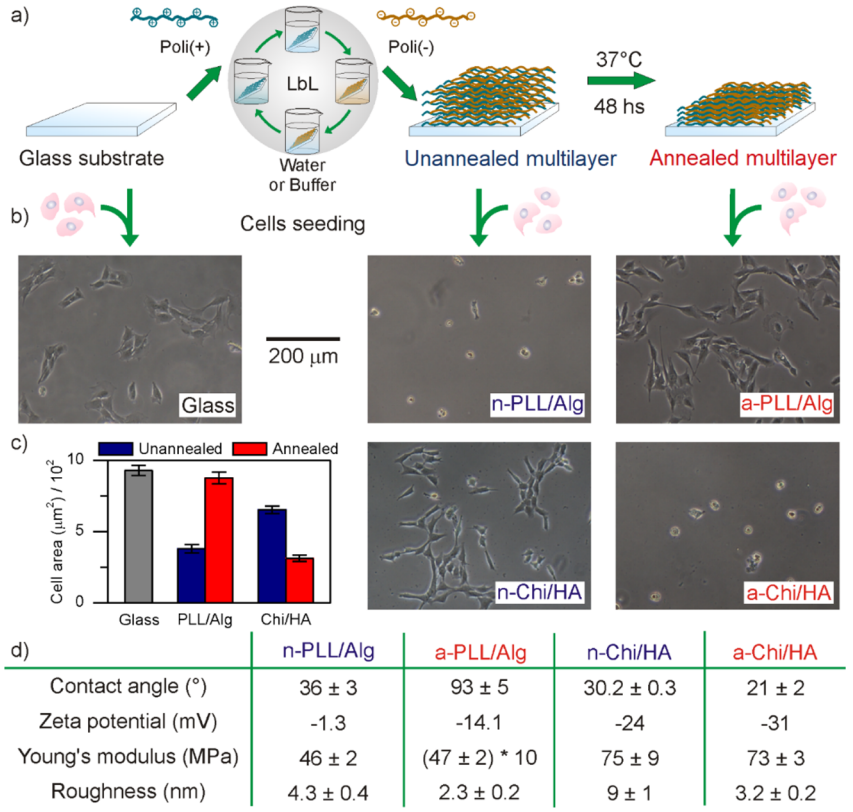

d)

	n-PLL/Alg	a-PLL/Alg	n-Chi/HA	a-Chi/HA
Contact angle (°)	36 ± 3	93 ± 5	30.2 ± 0.3	21 ± 2
Zeta potential (mV)	-1.3	-14.1	-24	-31
Young's modulus (MPa)	46 ± 2	(47 ± 2) * 10	75 ± 9	73 ± 3
Roughness (nm)	4.3 ± 0.4	2.3 ± 0.2	9 ± 1	3.2 ± 0.2

Figure 3. Changes in cell adhesion and in the physicochemical properties of layer-by-layer (LbL) multilayer coatings induced by thermal annealing.[157] (a) Scheme of the assembly and annealing protocols. (b) Phase contrast images of C2C12 cells adhered on glass, poly-L-lysine/alginate (n-PLL/Alg, a-PLL/Alg), n-chitosan/hyaluronic acid (n-Chi/HA), or a-Chi/HA as indicated. (c) Average cell adhesion spreading area from cells seeded on glass, n-PLL/Alg, a-PLL/Alg, n-Chi/HA, or a-Chi/HA polyelectrolyte multilayers. (d) Changes in physicochemical properties of polyelectrolyte multilayers upon annealing. Adapted with permission from ref 150. Copyright 2019 John Wiley & Sons, Inc.

cationic polymers that disrupt bacterial membranes,[189] to the assembly of antibacterial nanomaterials such as silver nanoparticles or graphene oxide,[190] to the encapsulation of antibiotics in the multilayers, to combinations of all these elements.[191] The LbL assembly can include several layers of nanomaterials, combine layers of different nanomaterials in a film, or facilitate inclusion of antibiotics in the films by complexing with the polymers (see Figure 1e). Many antibiotics have charged groups that can be used to form complexes with polyelectrolytes in the LbL films or assembled in films replacing polyelectrolyte layers. The LbL technique has the advantage that it can be applied straightforwardly on almost any charged surface, and antibacterial coatings could be developed for medical devices as well as for implants. In particular, the LbL technique has significant potential in the design of antibacterial coatings that can inhibit nosocomial infections during implant surgery. An optimal antibacterial coating for bone implants based on release of an antibiotic should involve an initial burst release at the time of surgery, followed by prolonged release over the weeks following the surgical intervention to ensure bone tissue regeneration.[192] The LbL technique can be used to design films capable of fully or partially degrading and releasing antibiotics at different times and rates. Examples in the literature show that aminoglycans, such as gentamicin, can be released from LbL films, combining burst and steady releases that would be particularly suitable for implant surgery.[193] Moreover, the LbL technique enables the additional assembly of growth factors on the coating that can counteract negative effects on cell growth and differentiation caused by a high localized dose of antibiotics.[194] These combinations can result in films with enhanced antibacterial properties and in the design of coatings suitable for different environments in multiple medical settings or for antifouling applications.

LAYER-BY-LAYER-BASED MEMBRANES

Purification Technologies. Another future for LbL coatings lies in separation technologies, such as liquid or gas permeation membranes (see Figure 4).[69,195,196] Significant pioneering work has already been carried out,[197−201] starting with gas separation membranes,[69] but recently, LbL membrane modification for fresh water production has been explored further. Nanofiltration membranes for the removal of particles down to virus sizes of ∼35 nm are not able to retain dissolved materials, such as ions, leading to issues with water hardness, low molecular weight pharmaceutical agents, *etc.*, which become increasingly problematic in fresh water preparation. However, reverse osmosis (RO) membranes consume a great deal of energy and retain all salts, which is not useful for drinking water. In contrast, a few LbL-assembled layers of poly(diallyldimethylammonium chloride) (PDADMAC)/PSS on top of tubular filtration membranes of pore size 20 nm are able to increase the retention of magnesium sulfate from 5% to over 90% and for several endocrines above 50−90% depending on the endocrine type (see Figure 5).[202] In contrast to RO membranes, these LbL membranes allow permeation of sodium chloride, maintain high fluxes, and require much less pressure and energy. Up to now, the LbL coating of membranes has been evaluated only for films based on the

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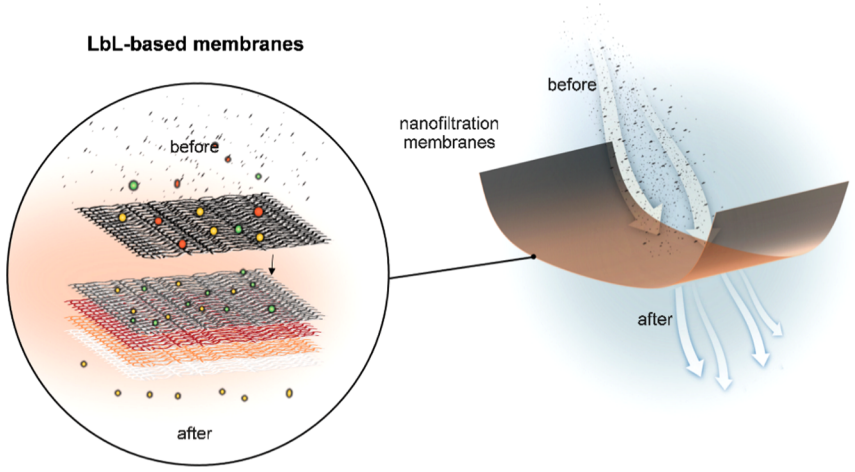

Figure 4. Layer-by-layer (LbL) assembly can be used for membranes for gases and liquids.

combination of one polycation with one polyanion. However, one can imagine that a multifunctional coating could improve the membranes further. The first layer on the membrane has to ensure a good connection of the LbL film to the membrane in order to resist sufficiently high-pressure backflushing cycles. Furthermore, the first polyelectrolyte has to be assembled exclusively on top of the pores and should not penetrate into the pores, as otherwise these would be blocked. The intermediate layers should utilize a design in which the mesh size controls the retention of the analyte and also ensures the removal of specific pollutants.[91] Finally, the outermost layer should reduce the fouling behavior of the membranes by controlling its hydrophilic properties and electrostatic repulsion.[203−205]

Introducing Channels in Biological Membranes. Biomimetic nature of LbL materials opens the possibility to replicate biological membranes. Cell membranes comprise not only lipids but also high protein content,[206] for example, transmembrane proteins that form channels for molecular transport into/out of cells. Lateral inhomogeneity is important. Here lies one big challenge for the future. To date, LbL structuring has predominantly only been possible perpendicular to the surface, *i.e.*, by variation of the compositions of the different layers. However, in order to create LbL-assembled membranes mimicking the function of biological membranes—for example, with integrated protein-based channels—lateral structuring would also be required. In the simplest case, "channels" in the form of holes could be introduced, for example, by nanoplasmonic heating.[207] Another option lies in tethered membranes. In recent work, dense membranes with limited defects and high resistivity were assembled on top of multilayers.[208−211] These membranes can contain channels with selective ion permeability.[212] For electronic sensing, the multilayers provide a means to control the distance of the lipid bilayer from the electrodes, which is particularly useful for membranes incorporating channels and transmembrane proteins, avoiding undesired effects from the electrode on channel and protein behavior.[212] Another method for lateral structuring might be based on the fusion of microcapsules.[213] Still, despite the numerous ideas outlined here, lateral structuring of LbL films remains a challenge.

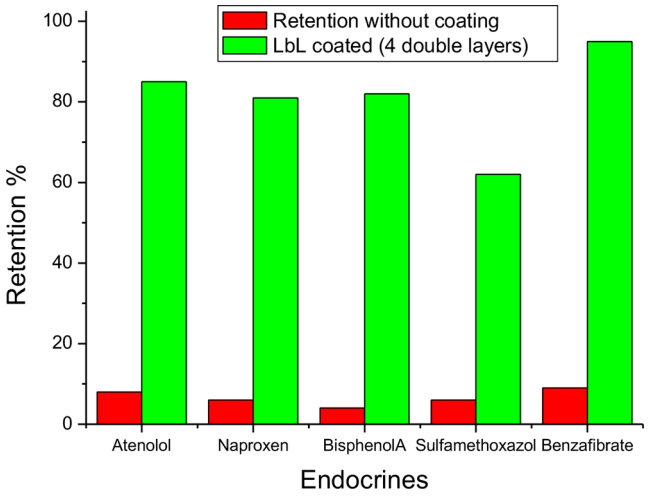

Figure 5. Retention of different endocrines by an uncoated poly(ether sulfone) membrane (red) and by a layer-by-layer (LbL)-coated $(PDADMAC/PSS)_4$ membrane (green). Unpublished data by the group of Lars Dähne.

LAYER-BY-LAYER-BASED ENCAPSULATION FOR DELIVERY VEHICLES

Layer-by-layer Assembly for Encapsulation. Layer-by-layer technology for micro- and nanoencapsulation was introduced ~20 years ago and initially looked extremely promising (see Figure 6).[10,11,214−219] The key advantage was considered to be the simplicity with which one could construct multifunctional delivery systems. In fact, LbL capsules can combine multiple functions and external responsiveness. However, LbL-based encapsulation suffers from high permeability of small, water-soluble molecules (*i.e.*, leaching) and rather time-consuming processes for fabrication. Some of these problems have been solved, such as expanding the class of molecules that can be encapsulated (*e.g.*, doxorubicin, paclitaxel, liquid crystals, siRNA) without severe leaching,[44,45,220−225] and inroads have been made into the problem of scale-up.[61,226,227] This approach has also been made possible by extending the initial capsule geometries to more sophisticated structures, such as capsosomes, *etc.*[228−235] The capsule shells can also be labeled with different types of nanoparticles, providing contrast for imaging[118−120] or enabling magnetic targeting.[236,237] Currently, the technology still has potential, particularly in areas where other technologies are not available. A number of studies on various cell types, including macrophages, dendritic cells, neurons, and stem cells, have

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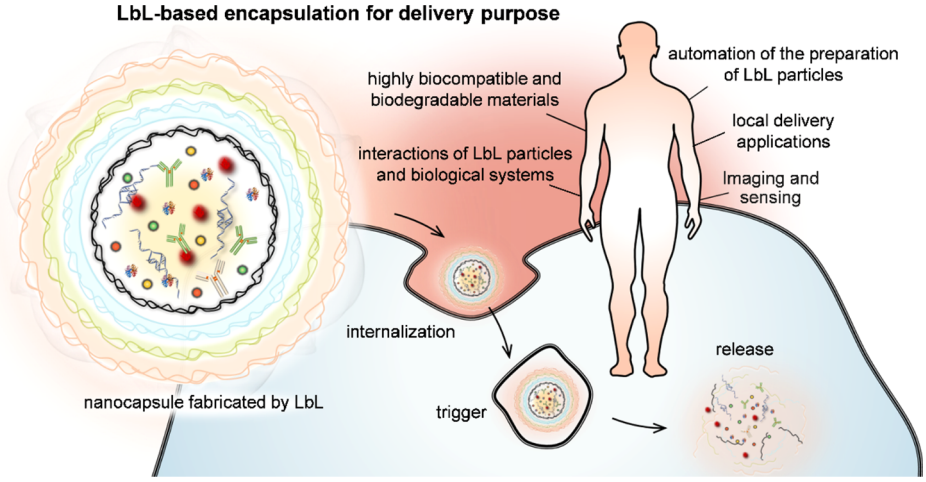

Figure 6. Layer-by-layer (LbL) assembly can be used to fabricate encapsulation platforms for nanodelivery.

demonstrated that incubation with cells results in internalization of capsules by cells without significant effects on cell viability.[238−240] The elastic properties facilitate their uptake as the capsules can easily be deformed during internalization.[241−244] In other words, cells were found to tolerate capsule internalization, which is not always the case for other delivery systems. Detailed studies on the tissue response after subcutaneous[245] and pulmonary[246] administration of degradable LbL capsules composed of polypeptide and polysaccharide building blocks have also demonstrated that LbL capsules exhibit a moderate foreign body response and are easily internalized by immune cells, such as macrophages and dendritic cells. This advance should pave the way for further development of such carriers in advanced vaccine technologies. In summary, LbL offers a good platform for delivery of encapsulated cargo inside cells, which is discussed below in terms of drug delivery and imaging/sensing. Because the capsules remain in endosomes/lysosomes after internalization, endosomal escape and translocation of encapsulated compounds to the cytosol remains a significant hurdle.

Delivery of Therapeutic Agents. The LbL technique opens the possibility of assembling therapeutics in between layers of polyelectrolytes, on top of nano/microparticles that protect a certain cargo, while, at the same time, making multiple functional groups available in the polyelectrolyte, which can be engineered to generate stealth coatings for targeting delivery. For the delivery of encapsulated therapeutics in polyelectrolyte multilayers, the assemblies must degrade, liberating the material entrapped between the layers. However, as we noted above, LbL assemblies have intrinsically semipermeable properties that can be tuned by means of layer numbers and thicknesses as well as by the type of the interacting polyelectrolyte pairs, resulting in leaching even before intended degradation and subsequent release. Thus, the initial euphoria in scientific articles to encapsulate low molecular weight drugs and to release them in a controlled manner on demand has not yet translated into real-world applications. As previously noted, the most critical reason for this difficulty in translation is the high permeability of the films for small molecules (see Figure 7). Even for the very dense polyelectrolyte system poly(allylamine hydrochloride)/polystyrenesulfonate (PAH/PSS), researchers recorded release rates ranging from minutes to a few hours for water-soluble molecules having molecular weights below 5 kDa.[247] In contrast, large molecules with molecular weights above 10 kDa can be permanently immobilized, either in the polyelectrolyte layers or in capsules comprising polyelectrolyte walls. This same conclusion has been shown over the past decade for a variety of biomolecules, including proteins, such as antibodies;[248] growth factors;[249] hormones;[250] enzymes;[251] nucleic acids, such as DNA plasmids;[237] different types of RNA molecules, such as silencing RNAs;[252] and polysaccharides such as alginate, carrageenan, chitosan, and hyaluronic acid.[253]

In parallel, new therapeutic avenues based on the delivery of high molecular weight drugs, for instance, at the site of the implantation of LbL material.[159] Plasmids, specific antibodies, RNA, or DNA can be delivered by incorporation in LbL films and can be utilized as personalized medicines. However, due to the sensitive and specialized recognition of such molecules by our immune system, it is hard to deliver them efficiently *in vivo* to the intended targets. For this purpose, LbL assemblies could have a bright future in the form of capsule formulations, because the necessary multifunctionality can be delivered by LbL technology. For example, an ideal capsule should have an inner surface that is not interacting with the biomolecule in order to retain its functionality. The intermediate layers determine the release behavior, which could be controlled slow release, immediate release caused by an internal trigger (*e.g.*, by

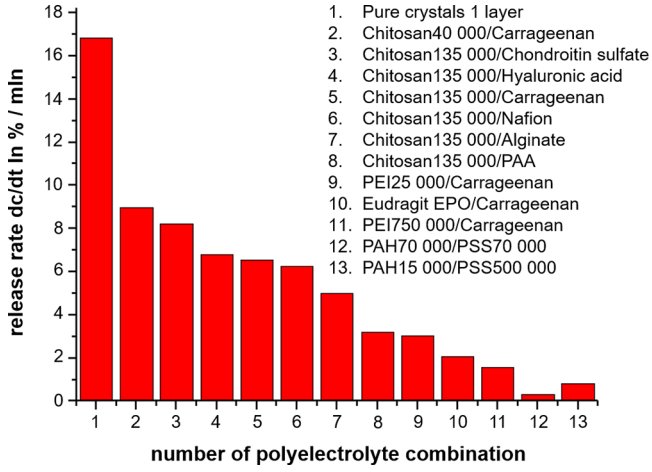

Figure 7. Permeability of layer-by-layer (LbL) membranes consisting of different polyelectrolyte combinations (8 layers) for small molecules (fluorescein). Unpublished data from the group of Lars Dähne.

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the lower pH value in cancer cells or by specific enzymatic surroundings[254,255]) or release activated by an external trigger (*e.g.*, NIR light,[22,237,256−258] X-ray radiation, ultrasound (US),[259−261] or magnetic fields[262,263]). Internal triggers can readily be created by combining polycations and polyanions in such a way that their degradation will be fast or slow. The sequential assembly of polyelectrolytes in LbL enables control over the composition of the layers in the vertical direction and could be used to deliver different therapeutics progressively. For example, two siRNA molecules with complementary actions could be assembled in different positions within the LbL film, so that they are released sequentially. Degradation of the multilayers in biological fluids or intracellularly can be selected because matrices can be programmed by varying the assembly conditions, the number of assembled layers and the combination of polycations and polyanions. The surface layer is also important: It should not be recognizable by the immune system in order to realize high circulation times, but it should nonetheless bind specifically to a defined target. In the case of systemic delivery, there is the problem of targeting, *i.e.*, to produce locally enhanced concentrations of the pharmaceutic agent at the desired target site. One interesting approach, which has not yet been fully exploited, is cell-mediated delivery, where cells are used as natural transporters to carry the encapsulated materials to a targeted site.[264] Here, externally driven cell navigation could be used.[265] *In vitro* studies have demonstrated that cell motion is possible in magnetic field gradients if the cell has internalized magnetic capsules.[266] Magnetic capsules can bring genetic materials inside the cells and reprogram the cells in such a way that the follow-up sorting of altered and nonmodified cells can easily be done by magnetic sorting.[267] Such magnetic targeting is biocompatible. The ability of mesenchymal stem cells (MSCs) to differentiate was not affected by magnetic manipulation.[268] This is important, as MSCs impregnated with capsules could be used as natural cargo transporters. Also, whereas many applications focus on systemic delivery, local delivery may offer new approaches, which deliberately avoid the "targeting" issue. One interesting example is LbL particles that were designed for transdermal delivery of vaccine and adjuvant peptides *via* hair follicles. In contrast to dissolved molecules, particles in sizes ranging between 300 and 900 nm can be inserted in hair follicles by intense massage.[269] The diffusion of vaccines to the Langerhans cells in the skin is much easier through the follicle membrane than through the epidermis. In order to transport the vaccine peptides into the hair follicle, 600 nm silica particles were coated with polymethacrylate with LbL assembly, with an outermost layer having a pK_a value of 6.2. The pH difference between skin (pH 5−5.5) and follicle center (pH 7.4) was selected for the delivery of the peptides, which were tagged with four glutamic amino acids bearing negative total charge. The vaccines were efficiently adsorbed at pH 4.5 onto the partly positively charged particles and kept stably attached during the skin massage. After arriving at the follicle center where the pH was 7.4, the zeta-potential of the particles switched and became highly negative and the vaccines were released due to electrostatic repulsion. In general, LbL capsules have made the step from *in vitro* demonstration to *in vivo* experiments. For example, these particle systems can induce bone formation *in vivo* (when loaded with growth factors),[249] target atherosclerotic plaques *in vivo*,[270] and generate a significant immune response *in vivo* (when loaded with immunogenic peptides).[271] Both peptide- and protein-antigen-loaded LbL capsules generate a significant immune response *in vitro* and *in vivo*.[254] It was demonstrated that ovalbumin (OVA) (a model vaccine)-specific CD4 and CD8 T cells were activated to proliferate *in vivo* following intravenous[271] and subcutaneous[272] vaccination of mice with OVA protein- and OVA peptide-loaded LbL capsules. The OVA encapsulated within the capsules resulted in greatly enhanced antigen presentation and proliferation of antigen-specific CD4 and CD8 T cells that provided enhanced protection against viral infection and tumor growth. Furthermore, LbL capsules could be further engineered on their surface with immune-stimulatory molecules to boost the antigen-specific immune responses against encapsulated antigen.[273] The latter work was carried out with the idea of using LbL-coated microneedles for transdermal vaccination. Indeed, several groups have investigated codelivery of antigen and immune stimuli, both on colloidal and planar substrates.[273−275]

Imaging and Sensing. In diagnostic imaging, highly developed methods/modalities are applied, such as ultrasound imaging (US), X-ray computed tomography (CT), magnetic resonance imaging (MRI), near-infrared imaging (NIR), photoacoustic imaging (PAI) and nuclear imaging methods such positron emission tomography (PET) or single photon emission computed tomography (SPECT). Each of these imaging methods has advantages, but also drawbacks, such as limited spatial or time resolution, sensitivity, impairment of the patient, *etc*. Therefore, several methods must be combined in order to optimize the images and information obtained.[276] Instruments for this purpose are already under development, but suitable contrast agents providing contrast for different imaging modalities and methods are also necessary. These agents can be based on molecular materials or on particles. Solid particles should be in the nanometer range, whereas flexible particles could be used at the micron scale of erythrocytes. By means of LbL technology, such multifunctional contrast agents can be produced in a controlled way. One example was recently developed, which is simultaneously applicable for US, MRI, SPECT, and NIR imaging.[277] The core of the flexible 3 μm particles consisted of an air bubble, which is stably encapsulated by cross-linked poly(vinyl alcohol) (PVA) for US imaging. Positive charges were introduced in the PVA matrix in order to achieve controlled LbL coating. Two double layers of PSS/PAH-1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) were assembled. The NOTA label complexes technetium for SPECT imaging. On top, double layers of citrate-stabilized iron oxide nanoparticles (SPION)/PAH were assembled for dark contrast in MRI imaging.[278] For NIR imaging, further fluorescent layers of PAH-Cy5/PSS were assembled. Finally, targeting was demonstrated by biotinylated antibodies coupled to an outermost PAH/streptavidin layer.[279,280] Thus, LbL enables convenient integration of different contrast modalities in one single particle. Apart from simple imaging, where contrast depends on the local concentration of contrast agent, functional imaging, *i.e.*, sensing, is possible. In this case, the signal of the contrast agent also depends on the local environment. There are several examples of encapsulated, analyte-sensitive fluorophores,[281−283] which enable the detection of local ion concentrations. The changes in environment must be taken into account when designing these multimodal particles. For example, many ion-sensitive fluorophores also respond to local pH, so one severe challenge concerning future *in vivo* applications is that particles will undergo massive local pH changes along their trajectories in the body, for example, upon endocytosis by macrophages. One solution might be to use more complex systems, such as sensors with distance-dependent

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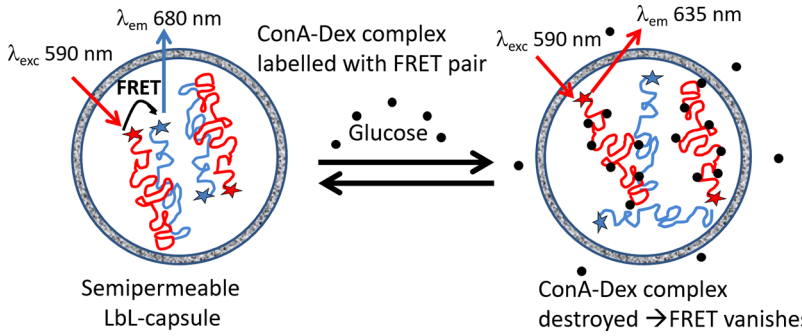

Figure 8. Glucose sensing in layer-by-layer (LbL) capsules, containing ConcanavalinA (ConA) and Dextran (Dex), labeled with fluorescence resonance energy transfer (FRET) pair. Unpublished data from the group of Lars Dähne.

quenching of optical or magnetic signals. In order to protect these systems from agglomeration, they could be encapsulated. The LbL shell around the sensors would then enable analytes to diffuse in and out, whereas it would retain and protect the actual sensor system. One developed glucose microsensor is depicted in Figure 8. Due to the possibility of multicompartment encapsulation of different molecules in different locations within one particle by LbL,[284] even feedback-controlled systems might be developed. A drug could be encapsulated for delivery in one compartment, whereas a sensor monitoring the action of the drug could be placed in an adjacent compartment.[285,286]

Challenges for Layer-by-Layer Coated Particles Intended for *in Vivo* Use. Following the above-outlined possibilities for applying LbL-based particles to *in vivo* delivery and imaging/sensing, one can summarize a number of key challenges for the future. (1) Highly biocompatible and biodegradable materials need to be developed and used. (2) Further fundamental studies need to be undertaken to understand the interactions of LbL particles and biological systems in order to probe parameters such as elasticity and shape and how these influence biological responses. (3) Automation of the preparation of LbL particles should be further developed, as this capability is critical to reproducibility and streamlining preparation. (4) More focus should be placed on such particles for local delivery applications (*e.g.*, their use as depots) and their interactions with the local cellular and protein environment, not only limiting their studies to systemic delivery applications. Thus, the development of LbL-based vehicles continues and important breakthroughs lie ahead.

By means of layer-by-layer technology, multifunctional contrast agents for diagnostic imaging can be produced in a controlled way.

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Notes

The authors declare no competing financial interest.

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DEDICATION

This article is dedicated to the memory of our beloved colleague and friend Prof. Dr. Helmuth Möhwald (19 January 1946–27 March 2018).

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